Current Biology, Vol. 12, R663–R665, October 1, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0960-9822(02)01162-4

ER Quality Control: A Function for Sugars in the Cytosol

Dispatch

J. Michael Lord and Lorenzo Frigerio

Misfolded glycoproteins in the endoplasmic reticulum of a eukaryotic cell are exported to the cytosol, where they are proteolytically degraded by the ubiquitin–proteasome system. A recent study has identified a novel E3 ubiquitin ligase that recognises target glycoproteins via their sugar moieties.

Many cellular processes are regulated by the degradation of key proteins. Proteolysis can occur in different ways, such as the protease cascades in apoptosis or in specialised cellular organelles of the lysosome/endosome system, but the degradation of most short-lived proteins in a eukaryotic cell is accomplished by the ubiquitin–proteasome system [1]. Processes regulated in this way include cell-cycle progression, transcription, signal transduction and endocytosis, and the system also functions in the elimination of aberrantly folded polypeptides. A recent study [2] has provided interesting new insights into the way in which misfolded glycoproteins of the endoplasmic reticulum (ER) are targeted for degradation by the ubiquitin–proteasome system.

The proteasome is a 26S protease complex found in both cytosol and the nucleus. Proteins targeted for proteasomal degradation are usually tagged with ubiquitin, a highly conserved, 76 residue polypeptide, which is covalently attached to lysyl residues of the doomed protein. Ubiquitination is catalysed by a series of enzymes: a ubiquitin activating enzyme (E1), a ubiquitin conjugating enzyme (E2), a ubiquitin protein ligase (E3) and, in some cases, a ubiquitin chain elongation enzyme (E4). The specificity of protein ubiquitination is conferred by the E3s, a diverse group of enzymes that recognise the target protein substrates. While some E3s are monomeric, most appear to be oligomers. One particular family of oligomeric E3s are known as SCF complexes for their subunits Skp1, Cdc53/Cullin and an F box protein, where the F box protein is an adaptor subunit that confers target substrate specificity [3]. A superscript denotes the identity of the F box protein: for example, Cdc4 is the F box subunit in the SCF^{Cdc4} complex. F box proteins come in many different forms: all have an approximately 45 residue F box domain, but they are otherwise very dissimilar. The F box domain binds to the Skp1 subunit in an SCF complex (Figure 1).

One important cellular process dependent on the ubiquitin–proteasome system is the ER-associated protein degradation (ERAD) pathway. Newly synthesised proteins in the ER of a eukaryotic cell are subject to a stringent quality control system. Those proteins that fold correctly, or are assembled correctly into appropriate oligomers, are retained in the ER or allowed to proceed to their ultimate destination; but those that do not fold correctly, and orphan subunits of oligomers, are destroyed by ERAD. Such failed proteins are exported across the ER membrane into the cytosol, where they are ubiquitinated and degraded by the proteasome.

Many proteins in the ER are N-glycosylated, and the glycans interact with resident ER lectins allow -ing the newly synthesised glycoprotein to enter the calnexin–calreticulin folding cycle [4]. Glycoproteins that fail to reach their native conformation are eliminated by ERAD. Precisely how they are selected for ERAD remains unclear, but what is now clear is that trimming of the N-glycans plays a key role in the

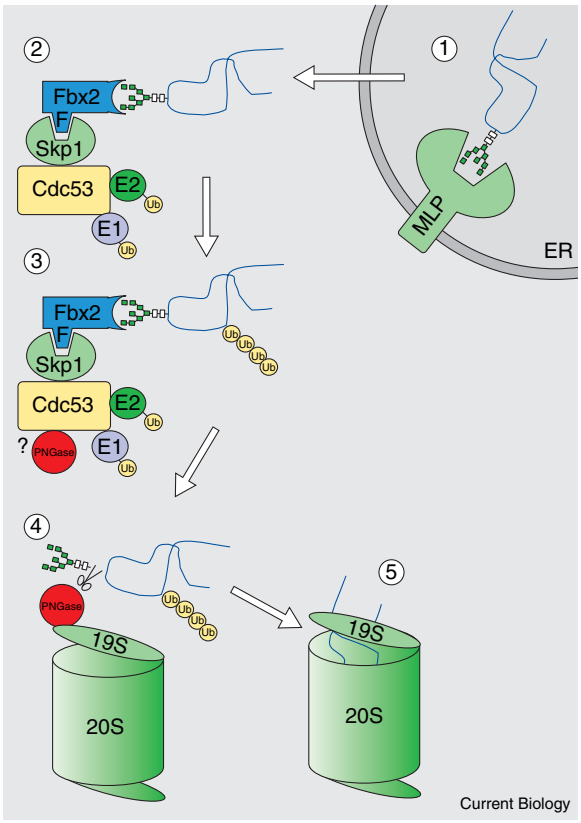

Figure 1. A model for the role of N-linked glycans in ERAD.

(1) A glycoprotein with permanent structural defects and carrying a glycan in the form $Glc_{0-3}Man_8GlcNAc_2$ is released from the calnexin–calreticulin cycle [5], recognised by a mannosidase-like protein (MLP) and eventually dislocated from the ER to the cytosol. (2) Here the protein binds to the glycan-specific F box protein Fbx2 and is targeted to the SCF^{Fbx2}-type E3 ubiquitin ligase complex. (3) This results in the protein being polyubiquitinated. (4) The protein is subsequently deglycosylated by a cytosolic peptide N-glycanase (PNGase) that is closely associated with the ubiquitin ligase complex and/or the 19S lid of the proteasome. (5) Finally, the ubiquitinated, deglycosylated protein is degraded by the proteasome.

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK.

selection process [5]. In particular, recent evidence indicates that defective glycoproteins can be targeted to ERAD by the mannosylation state of their sugar chains. Recognition by a mannosidase-like protein in the ER [6,7] decrees that the glycoprotein be dislocated from the ER to the cytosol (Figure 1), where it can be ubiquitinated and targeted to the proteasome for degradation. In some cases, a cytosolic peptide N-glycanase removes the sugar chain [8]. It is not clear whether the sugars play any role once the condemned protein appears in the cytosol; it is also not known whether deglycosylation precedes, follows or is simultaneous with ubiquitination.

Yoshida and coworkers [2] have now found one of the missing links between glycosylation and ubiquitination. They have identified a ubiquitin ligase complex that specifically binds N-glycosylated proteins, leading to their ubiquitination. When mouse brain extracts were mixed with GlcNAc-terminated fetuin (GTF)-immobilised beads, two of the proteins that bound were specifically eluted with *N,N'*-diacetylchitobiose (chitobiose), but not with EDTA/EGTA or N-acetylglucosamine. Amino acid sequence analysis identified these proteins as Skp1 and the F box protein Fbx2, two components of the E3 ubiquitin ligase complex, SCF^{Fbx2}. Fbx2, but not Skp1, was found to interact with GTF, indicating that Fbx2 mediates the interaction between Skp1 and GTF.

The GTF-binding property of Fbx2 was mapped to the whole carboxy-terminal domain (residues 95–296). Fbx2, in common with other F box proteins, forms a complex with Skp1, Cul1 and Roc1, and the recombinant SCF^{Fbx2} complex was shown to ubiquinate GTF. Ubiquitination of GTF did not occur in the absence of SCF^{Fbx2} or with a complex containing the different human F box protein βTrCP1, and it was inhibited by chitobiose and abolished when GTF was deglycosylated using peptide N-glycanase. Collectively, these data show that SCF^{Fbx2} is an E3 ubiquitin ligase that recognises target proteins that are N-glycosylated.

In an overlay assay, several endogenous high-mannose-containing mouse brain glycoproteins bound to Fbx2. The major endogenous Fbx2-binding glycoprotein had a molecular weight of 120 kDa and was identified as integrin β1. Binding of endogenous proteins was inhibited by chitobiose, or by treating the proteins with endo-β-*N*-acetylglucosaminidase H — which cleaves high-mannose type oligosaccharides — and was completely abolished by peptide N-glycanase treatment. While Fbx2 bound to both galactose-terminated asialofetuin and GTF, it bound more effectively to mannose-terminated fetuin and ribonuclease B, which has a high mannose-containing oligosaccharide. Clearly, Fbx2 binds to sugars, most effectively to mannose.

Because high-mannose oligosaccharide-modified proteins are found in the ER, it seems likely that SCF^{Fbx2} is important in ER quality control in neurons, with a role in the ubiquitination and degradation of ERAD substrates. Supporting this contention, known ERAD substrates — a mutant form of the cystic fibrosis transmembrane conductance regulator (CFTR) lacking phenylalanine 508 [9] and the T-cell receptor α subunit [10] — were both shown to bind Fbx2, and they were found to be rapidly degraded in pulse-chase experiments. Because mutant Fbx2 with the F box domain deleted was shown still to interact with the ERAD substrates, it was assumed that overexpression of the mutant protein acts as a dominant-negative repressor of ERAD, and this was experimentally confirmed. These findings strongly suggest that SCF^{Fbx2} is specifically involved in the ubiquitination and degradation of misfolded glycoproteins that are translocated from the ER to the cytosol in neurons. Glycan-mediated elimination of ERAD substrates in other cell types may depend on SCF ubiquitin ligase complexes with different, as yet unidentified, F box proteins.

Post-translational modification is increasingly emerging as a prerequisite for targeting proteins to the ubiquitin pathway. Specific ubiquitin ligases recognise protein modifications, such as phosphorylation and prolyl hydroxylation [11,12]. The new work of Yoshida and colleagues [2] shows that N-glycosylation also plays a role in targeting ERAD substrates to the ubiquitin–proteasome pathway. Specificity is conferred by the E3 ligase complex.

Finally, if glycans are important for interaction with the E3 ligase complex, where does peptide N-glycanase, which removes glycans from proteins, fit into the picture? A recent study [13] based on use of the yeast two-hybrid assay has shown that cytosolic peptide N-glycanase interacts with several proteins, including ubiquitin, proteasome-interacting proteins and proteasome subunits. It is possible to envisage a scenario (Figure 1) in which the sugars serve as a signal resulting in ubiquitin attachment, only being removed by the glycanase after ubiquitination has occurred and degradation by the proteasome is inevitable.

References

1. Hershko, A. and Ciechanover, A. (1998). The ubiquitin system. Annu. Rev. Biochem. *67*, 425–479.
2. Yoshida, Y., Chiba, T., Tokunaga, F., Kawasaki, H., Iwai, K., Suzuki, T., Ito, Y., Matsuoka, K., Yoshida, M., Tanaka, K., *et al*. (2002). E3 ubiquitin ligase that recognizes sugar chains. Nature *418*, 438–442.
3. Patton, E.E., Willems, A.R. and Tyers, M. (1998). Combinatorial control in ubiquitin-dependent proteolysis: don't Skp the F-box hypothesis. Trends Genet. *14*, 236–243.
4. Helenius, A. and Aebi, M. (2001). Intracellular functions of N-linked glycans. Science *291*, 2364–2369.
5. Ellgaard, L., Molinari, M. and Helenius, A. (1999). Setting the standards: quality control in the secretory pathway. Science *286*, 1882–1888.
6. Hosokawa, N., Wada, I., Hasegawa, K., Yorihuzi, T., Tremblay, L.O., Herscovics, A. and Nagata, K. (2001). A novel ER alpha-mannosidase-like protein accelerates ER-associated degradation. EMBO Rep. *2*, 415–422.
7. Jakob, C.A., Bodmer, D., Spirig, U., Battig, P., Marcil, A., Dignard, D., Bergeron, J.J., Thomas, D.Y. and Aebi, M. (2001). Htm1p, a mannosidase-like protein, is involved in glycoprotein degradation in yeast. EMBO Rep. *2*, 423–430.
8. Suzuki, T., Park, H., Kitajima, K. and Lennarz, W.J. (1998). Peptides glycosylated in the endoplasmic reticulum of yeast are subsequently deglycosylated by a soluble peptide: N-glycanase activity. J. Biol. Chem. *273*, 21526–21530.
9. Johnston, J.A., Ward, C.L. and Kopito, R.R. (1998). Aggresomes: a cellular response to misfolded proteins. J. Cell Biol. *143*, 1883–1898.
10. Yu, H., Kaung, G., Kobayashi, S. and Kopito, R. (1997). Cytosolic degradation of T-cell receptor chains by the proteasome. J. Biol. Chem. *272*, 20800–20804.
11. Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., Asara, J.M., Lane, W.S. and Kaelin, W.G. Jr. (2001). HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science *292*, 464–468.

12. Deshaies, R.J. (1999). SCF and Cullin/Ring H2-based ubiquitin ligases. Annu. Rev. Cell Dev. Biol. *15*, 435–467.
13. Park, H., Suzuki, T. and Lennarz, W.J. (2001). Identification of proteins that interact with mammalian peptide:N-glycanase and implicate this hydrolase in the proteasome-dependent pathway fro protein degradation. Proc. Natl. Acad. Sci. U.S.A. *98*, 11163–11168.